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DOI:
10.1098/rspb.2018.2418

Rapid morphological evolution in placental mammals post-dates the origin of the crown group

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SUMMARY

Background
Resolving the timing and pattern of early placental mammal evolution has been confounded by conflict among divergence date estimates from interpretation of the fossil record and from molecular-clock dating studies. Despite both fossil occurrences and molecular sequences favouring a Cretaceous origin for Placentalia, no unambiguous Cretaceous placental mammal has been discovered. Investigating the differing patterns of evolution in morphological and molecular data reveals a possible explanation for this conflict. Here, we quantified the relationship between morphological and molecular rates of evolution. We show that, independent of divergence dates, morphological rates of evolution were slow relative to molecular evolution during the initial divergence of Placentalia, but substantially increased during the origination of the extant orders. The rapid radiation of placentals into a highly morphologically disparate Cenozoic fauna is thus not associated with the origin of Placentalia, but post-dates superordinal origins. These findings predict that early members of major placental groups may not be easily distinguishable from one another or from stem eutherians on the basis of skeleto-dental morphology. This result supports a Late Cretaceous origin of crown placentals with an ordinal-level adaptive radiation in the early Paleocene, with the high relative rate permitting rapid anatomical change without requiring unreasonably fast molecular evolutionary rates. The lack of definitive Cretaceous placental mammals may be a result of morphological similarity among stem and early crown eutherians, providing an avenue for reconciling the fossil record with molecular divergence estimates for Placentalia.

KEYWORDS
Placentalia, Evolution, Morphology, Molecular Clock, Palaeontology, Rate

INTRODUCTION

The Cretaceous fossil record of eutherian mammals – those more closely related to Placentalia than to the sister group Metatheria (which includes the extant marsupials) – is rich and diverse [1]. The earliest putative eutherian is from the Jurassic of China [2] (but see [3, 4]), with later Cretaceous eutherians known from North America, Asia, Europe, and the Indian subcontinent [1, 5-7]. Despite extensive fossil collection effort and phylogenetic analysis, the eutherian crown

group, Placentalia, has no unambiguous representative from earlier than the earliest Paleocene [8]. Although both molecular data and fossil occurrence patterns predict that placental mammals should be present in Cretaceous beds [9, 10], as yet no demonstrably Cretaceous placental fossil has been found. *Protungulatum*, known from the latest Cretaceous and Paleocene of North America [6], is the best candidate, having been recovered as a relative of extant ungulates in some analyses [11], but others have concluded that it too is a stem eutherian [8]. If this latter conclusion is valid, substantial ghost lineages exist between the earliest definitive placentals from the earliest Paleocene and the middle-Cretaceous molecular date estimates of the placental origin [12, 13], whether in a 'long fuse' model, where interordinal divergences in the Cretaceous predate a near-Cenozoic intraordinal divergences, or a 'short fuse', in which both inter- and intraordinal divergences occurred early in the Cretaceous [14]. The third major model, an 'explosive' model, whereby both inter- and intraordinal divergences occurred near the Cretaceous-Paleocene boundary, can further be characterised as 'hard' (i.e. all divergences postdating the end-Cretaceous mass extinction) or 'soft', where all divergences occur in a distribution surround the end-Cretaceous mass extinction but, unlike the hard model, can occur before the boundary itself [15]. The 'soft' model therefore retains aspects of both long and short fuse models, depending on the number and phylogenetic position of nodes reconstructed as diverging in the Cretaceous, but remains fundamentally different from either.

The apparent absence of placentals from the Cretaceous has generated much debate. If placental mammals originated in some currently unsampled region prior to a Cenozoic dispersal, the lack of Cretaceous placentals might be due to biases in the fossil record. A southern hemisphere origination has been often proposed [16, 17] but also refuted [18], with the only unambiguous Gondwanan Cretaceous eutherians, from India, resolved as stem eutherians [19]. Moreover, sampling of northern hemisphere localities is sufficiently dense that we would expect to have found Cretaceous placentals were they to have existed there [20]. Available fossils are of equal quality (in terms of scorable phylogenetically informative characters) in the Cretaceous as in the earliest Paleocene [21]. The only remaining hiding places for an 'off-camera' diversification of placental mammals are within unpreserved environments in the northern hemisphere; some environments are not conducive to fossilisation [22]. A placental diversification in an erosional montane environment or a rainforest landscape in which organic remains are rapidly decayed would be likely to be missed. Such are the possibilities from the fossil record being an unreliable narrator of placental mammal history. Alternatively, our modelling of molecular evolution may be misleading. Indeed, there is evidence for a rapid diversification of placental mammals in molecular sequences with the both the three-way split between Boreoeutheria, Afrotheria, and Xenarthra [23, 24] and the ordinal divisions within Laurasiatheria [25] particularly difficult to resolve. In each case, this difficulty suggests some degree of incomplete lineage sorting [26] or, at the very least, little time in which to accumulate and fix mutations. Indeed, there is strong evidence for substantial incomplete lineage sorting in certain transposable elements thought to have very low homoplasy [27].

One approach that may be fruitful when considering the apparent discrepancy between morphological and molecular data is to examine the relationship between

the patterns of evolution in these two types of data. A prediction of the neutral theory of molecular evolution [28] is that rates of morphological and molecular evolution should be decoupled, which has often been demonstrated [29, 30]. This expectation is sensible for several reasons: molecular sequences, though influenced by both drift and selection [28], are removed from the direct effects of natural selection, meaning that there is not a one-to-one correspondence between genotype and phenotype. Indeed, in mammals, large swathes of genomic architecture appear to evolve neutrally [31]. Conversely, exposure of phenotypic traits to direct selection is thought to contribute to both pulses in evolutionary rate and convergence to superficially similar morphologies. Moreover, molecular sequences are a very different type of data from morphological characters, composed of recognisable, independent subunits that evolve in an easily modelled way. Conversely, it is challenging to identify separate morphological traits that are, from a modelling perspective, equivalent. Nonetheless, morphology is our sole source of data for most extinct clades, and as such contains unique information about macroevolutionary patterns [32], and neither non-equivalence nor lack of good models entirely prevents important information from being recovered. For example, rates of morphological evolution are correlated with species diversification in multiple clades [33, 34]. Even small numbers of extinct taxa in macroevolutionary studies have a beneficial effect, and there is little justification for excluding a taxon on the basis of missing data alone [32, 35, 36]. Without fossils, we would be unable to reconstruct patterns of extinction, and would not be able to incorporate entirely extinct clades when modelling diversity, disparity, rates of evolution, and biogeography through time. For these reasons, identifying the ways in which morphological and molecular rates of evolution co-vary across phylogeny can therefore give important insight into a wide variety of macroevolutionary questions.

Although determining the absolute value of a rate of any kind requires divergence dates for the internal nodes of the tree, comparison of morphological and molecular rates of evolution does not. For any given branch, the time component of the evolutionary rate is shared between the morphological and molecular partition. The ratio of morphological:molecular branch lengths is therefore equivalent to the ratio of morphological:molecular rates of evolution. By calculating the ratio of evolutionary change for every branch on a phylogeny, the structure of the relationship between the two data sources can be established. A general null hypothesis might be that morphological and molecular evolutionary rates track one another through time, in which case the ratio is expected to be identical for all branches on the tree. Where deviations occur, we can identify branches in which more or less morphological change occurred than would be expected given clock-like molecular evolution. In the case of the placental mammal diversification, the observed burst in morphological diversity immediately following the end-Cretaceous mass extinction leads us to expect that such deviations should exist. By comparing the observed ratios of morphological:molecular evolutionary rate on each branch of the phylogeny with the expectation under this null model, we can identify evidence for increased rates of morphological change, and provide evidence for one or more models of placental diversification.

Branch ratios can only be computed for branches leading to extant taxa, as these have available molecular sequence data. However, exclusion of extinct taxa substantially changes interpretation of morphological trait evolution [37]. The diverse extinct placental mammal clades are still informative as to the patterns of character acquisition. We conducted a total-evidence phylogenomic analysis of placental mammal relationships including a large sample of Cretaceous and Palaeogene eutherians, calculated ratios of morphological and molecular evolutionary rates, and assessed their structure both on the phylogeny and with respect to a variety of previously dated phylogenetic timetrees.

METHODS

Taxon sampling and multiple sequence alignment of mammal genes

We downloaded nuclear protein-coding sequences (CDS) for all 43 mammal genomes available in Ensembl v. 83 using Ensemble BioMart [38]. The Ensemble database includes comparative genomic information about the orthology and paralogy of each protein-coding gene [39]. We only selected genes identified as having one-to-one orthology between human and the other 42 mammal species. For each protein-coding gene, we selected the associated canonical transcript sequence for our dataset (defined by Ensembl as the longest transcript without stop codons, and therefore including all exons). We downloaded mitochondrial genomes for 38 (of 43) species that were available in NCBI RefSeq [11]. Only the 12 protein-coding genes on the heavy strand were included for analysis. We removed sequences that contained STOP codons or had a mismatch between CDS and amino acid protein sequence data as deposited in Ensembl, that were not present in the mouse genome, that were present in fewer than ten of the 43 species, or where the length of the human gene was shorter than 100 codons.

The amino acid sequences of nuclear and mitochondrial genes were aligned using PRANK [40] with the rooted Ensembl tree as the guide tree. CDS alignments were generated from the amino acid alignments using pal2nal [41]. For each gene alignment, we estimated its phylogenetic tree by maximum likelihood using RAxML v. 8.2.4 (GTRGAMMA; [42]). We removed those genes where a single branch length was greater than 60% of the total tree length [12, 43]. After this procedure, our dataset contained 15,306 nuclear and 12 mitochondrial genes, comprising a total of 43,167,984 sites. By using only the high-quality Ensembl data, and establishing stringent and conservative criteria for including gene alignments, our data set avoids many of the problems derived from poorly-aligned sequence data [e.g. 44].

Detailed information about genes in the dataset is available in Supplementary Material File 1.

Taxon sampling and character coding of the morphological dataset

We modified a previously published data matrix of 177 genera, [8] primarily sampling Palaeogene and Cretaceous eutherian mammals, to include an additional 58 extinct and extant taxa. 3 of the 43 genera for which genomic data were sampled were already included in the data matrix (*Procavia*, *Pteropus*, and *Tupaia*); we coded the remaining 40 for available morphological characters. In addition, we expanded the sample of rodents, xenarthrans, and South American Native Ungulates such that we had a diversified sample of Cretaceous and

Cenozoic eutherian families. Two further important extinct taxa were included – the Cretaceous Indian eutherian *Deccanolestes* [5] and the early afrotherian, *Ocepeia* [45].

Current phylogenetic software implementations do not correctly account for ascertainment bias (removal of parsimony-uninformative characters) for greater than 2 morphological character states (pers. obs. ZY). Each multistate character of Halliday *et al*. [8] was therefore separated into two or more binary characters prior to further coding, expanding the character number to 748. Ordered characters cannot be reasonably split without being hugely non-independent. We reduced the number of states within such characters to two by combining states within the sequence, defining the break as the one which resulted in the most even division of taxa. For example, if a character had three states, 'above' (represented by 20 taxa), 'equal' (represented by 40 taxa) and 'below' (represented by 30 taxa), the new character would have states 'above or equal to' and 'below'. Unordered multistate characters were split such that trait presence/absence was scored separately if applicable, while characters composed of multiple, associated observations were split into their component parts.

Phylogenetic analysis

Because third codon positions are frequently saturated in genome-level analyses, and to reduce the data set to a manageable size for computation, third codon positions were removed from the molecular alignment, and morphological and molecular alignments were concatenated in phylip format.

Maximum likelihood phylogenetic analysis was conducted in RAxML v. 8.2.9 using the UCL computer cluster Legion. Due to known issues where taxa can be resolved into different clades on the basis of structured missing data [46], we implemented a constraint on the position of several unambiguous members of extant lineages (Supplementary Information File 2).

Computing restrictions prevented simultaneous analysis of the entire genomic data set along with morphological data, so multiple replicates of subsampled molecular data were conducted. Preliminary tests indicated that a molecular sample of 150,000 sites was more than sufficient to recover consistent relationships among the 43 taxa with molecular data. Phylogenetic analyses were conducted on 1,000 independent molecular subsamples, each combined with the morphological data. The morphological partition was analysed under the binary Mk model [47], correcting for ascertainment bias, while the molecular partition was analysed under the GTR+G4 model.

Relative rates of evolution

We pruned each tree to the 43 taxa with molecular data, giving a standard molecular tree with the addition of morphological branch lengths incorporating information from extinct clades. The only differences topologically among the pruned trees concerned the position of Chiroptera, which had been rendered unstable within Laurasiatheria by the ambiguous positions of several extinct lineages. The 1,000 trees were split into three groups of trees dependent on the position of Chiroptera. In each case, we scaled partitioned molecular and morphological branch lengths as a proportion of the maximum value within the

tree, and calculated ratios between the morphological and molecular branch lengths.

Rates of evolution through time
To place the relative rates in a macroevolutionary context, we used four previously published time trees of mammalian relationships derived from three different dating methods: a stochastic fossil occurrence model [10], and three analyses of molecular data using varied methods [12, 13, 15]. In each case, we pruned the trees down to the same branches, and calculated rates of evolution, and the ratios between them, during each time bin.

RESULTS/DISCUSSION

Branch length ratios on the tree
Uncertain placement of several extinct clades, particularly of enigmatic Paleocene taxa, has been noted in several previous analyses [8, 48, 49], and was not improved here. The morphological data continue to include only weak phylogenetic information about many groups, so that RAxML analysis of morphological data alone, constrained to the comparatively well-supported molecular tree, produces many nearly equally good best trees. The maximum likelihood trees in the analysis of the morphological and subsampled molecular data therefore vary substantially across the 1,000 replicates (Figure 3). Regardless of this, the estimated rates of evolution in both partitions, and the calculated ratios between partitions, do not substantially change; our results are robust to the topological uncertainty in the data set. On an extant-only analysis, the sample size of 150,000 base pairs was sufficient to consistently reconstruct the phylogeny. This consistency in relationships makes comparison between branches possible. Although there are substantial differences in the order of divergence of extinct lineages from the internal branches leading to extant clades, these differences do not extend to the overall pattern of morphological:molecular length ratios.

Those morphological:molecular ratios on branches leading to Placentalia, Boreoeutheria, Laurasiatheria, Scrotifera, and Euarchontoglires are all exceptionally small (Fig. 1); very little morphological evolution is predicted to have occurred on those lineages relative to the amount of molecular evolution. By contrast, the branches leading to Atlantogenata, Euarchonta, Glires, Carnivora, Chiroptera, and to the rest of Scrotifera (as well as those leading to the great apes and haplorhines) are longer than would be expected (Fig. 1), suggesting relatively high rates of morphological evolution relative to molecular changes. Some of the larger ratios are explicable by known patterns of variation in molecular rates of evolution; slower molecular rates in apes, especially humans, have been documented [50]. This reduction in molecular evolution would, all else being equal, lead to a higher ratio of morphological:molecular evolution. Absolute values of molecular and morphological branch lengths suggest that the high morphological:molecular ratio for Xenarthra is here primarily due to exceptionally low values of molecular evolution, a phenomenon already observed to a lesser extent in this clade [50]. Absolute morphological branch length for Xenarthra is comparable to, for instance, that leading to Glires, and smaller than many other branch lengths. However, the molecular branch length is among the shortest. If molecular rates better reflect durations of branches, this implies a large increase

in morphological rate in a short period of time. One possible explanation is the preponderance of dental characters, which are highly simplified or lost in xenarthran dentition [51, 52].

It has frequently been observed [e.g. 53] that estimated molecular branch lengths between outgroup and ingroup taxa are shorter than expected. That pattern is seen here in the molecular branch lengths (Figure 1B, C). In morphological data, few consistent patterns have been observed [54, 55]. Given that the known bias would give a smaller denominator, our expected results would be that the morphological:molecular rate would be biased to be *higher*. As we observe the reverse, we can be confident that there were indeed relatively low rates of morphological evolution relative to molecular rates in the early placental lineages.

Branch length ratios through time

Comparing these ratios to previously dated timetrees, interesting patterns emerge (Figure 2). Using timetrees derived from the stochastic cal3 method [10], which incorporates fossil occurrence times in estimating rates of speciation, extinction, and sampling [56], those early branches with especially low morphological:molecular ratios are entirely within the Cretaceous. The branches that cross the end-Cretaceous mass extinction lead to Atlantogenata as well as Euarchontoglires, and, depending on the reconstruction, certain divisions within Laurasiatheria. These broadly match those internal branches that here consistently have higher relative rates of morphological evolution. A similar result is true of the geomolecular timetree [15], except that Atlantogenata diverges earlier.

In the Bayesian phylogenomic timetree [12], more deeply-nested branches cross the K/Pg boundary. Those lead to Paenungulata and Afroinsectiphilia within Afrotheria, to Cingulata and Pilosa within Xenarthra, to Eulipotyphla, Artiodactyla, Ferae, Perissodactyla, and Chiroptera within Laurasiatheria, and to Lagomorpha, Rodentia, Strepsirrhini and Haplorrhini, Dermoptera, and Scandentia within Euarchontoglires. The margin of error for Marsupialia also intersects with the K/Pg boundary. In this case, the branches with high rates within Laurasiatheria are also associated with the end-Cretaceous mass extinction, but those within Euarchontoglires and Atlantogenata predate the extinction event.

The timetree of Meredith *et al.* [13] shows a similar pattern. Much of the interordinal diversification is reconstructed as having occurred in the Cretaceous, with divisions occurring in Afrotheria, Afrosoricida, Eulipotyphla, Chiroptera, Primates, and Rodentia prior to the end-Cretaceous mass extinction. Again, those branches leading to Laurasiatherian orders largely intersect with the K/Pg boundary and have high morphological:molecular rates of evolution, while the high rates on branches leading to Atlantogenata, Euarchonta, and Glires are recovered in the Late Cretaceous.

Discussion

The implications of this structured pattern of morphological and molecular evolutionary rates are wide-reaching. Irrespective of the correct dates of the early placental nodes, the estimated amount of morphological change per fixation in the early divergences of placental mammals was very low. As the initial diversification of placental mammals did not include a substantial shift in rates of morphological

change, the idea that an intrinsic ‘key innovation’ permitted adaptive radiation is implicitly refuted.

The independent jump to high rates of morphological evolution per mutation in multiple lineages (Fig. 1) strongly suggests that some external factor was the common cause behind placental diversification. Although recent attempts to date the phylogeny of placental mammals have been sensitive to priors, methods, and data, the most recent examples of phylogenomic, molecular clock, and fossil occurrence-based methods all predict that the origins of the Laurasiatherian orders and of the superorders were close to the end-Cretaceous mass extinction, often with error bars encompassing that event [12, 13, 15, 57]. The end-Cretaceous mass extinction remains the most plausible candidate for that extrinsic cause, and, given the variation in rate required for a hard explosive model of placental mammal evolution to be viable [58], is not unlikely.

If placental morphological evolution increased relative to molecular evolution, this might explain aspects of the conflict between molecular and fossil-based estimates of the origin of Placentalia and its orders. If little morphological change occurred during the initial diversification of Placentalia, it follows that early placentals should be difficult to distinguish from stem eutherians. Indeed, this should also be true of early scrotiferans, eulipotyphlans, and euarchontoglires. If these three were morphologically very similar to one another and to late stem eutherians, the lack of definitive crown placental mammals in the Cretaceous is plausibly explained by a lack of characters by which to distinguish them. We might have already found Cretaceous crown placental mammals without being able to unambiguously identify them – leptictids, *Protungulatum*, and several ‘cimolestids’ are commonly reconstructed as close relatives of the crown group [8, 59], and are part of the same region of anatomical morphospace [60]. All survived into the Palaeogene and are certainly part of the story of eutherian diversification. We have already demonstrated changes in absolute rates of morphological evolution associated with this time period rather than with any particular clade [10], and that pattern is also seen here with relative rates of evolution. The origin of placental mammals cannot be directly associated with rapid morphological change, in absolute or relative terms, and the burst of morphological diversification observed in the fossil record is best associated with the ordinal-level diversification within Boreoeutheria, and at the level of superorder in Atlantogenata.

We know independently from palaeontological and molecular studies that the origin of Placentalia was almost certainly in the Late Cretaceous. On the basis of our results and previous dating from the fossil record [1], we favour the hypothesis that a changing balance between intraspecific selection pressure and interspecies competition caused the adaptive radiation of placental mammals. During the Late Cretaceous, speciation generated four major placental lineages – the ancestors of Atlantogenata, Euarchontoglires, Eulipotyphla, and Scrotifera. Until the end-Cretaceous mass extinction, rates of morphological evolution were low. The extinction of 75% of terrestrial life, including a disproportionate number of large-bodied organisms [e.g. 61] reduced niche occupancy, and hence interspecific competition and associated selection pressures [62, 63]. In the extinction’s aftermath, release of ecological constraints and higher niche availability allowed

morphological diversification of these four lineages. With more avenues in the adaptive landscape through which to evolve (as a result of empty niches), the phenotypic result of any given mutation would be more likely to be beneficial *in some direction* (that is, into an empty niche), leading to an increased proportion of mutations likely to be driven to fixation by selection pressures.

Each lineage crossing the K-Pg boundary evolved and speciated rapidly, with eutherians, like metatherians, quickly exploring ecomorphospace [60, 64]. Scrotifera diversified particularly quickly, resulting in the complex patterns of relationships associated with incomplete lineage sorting within the clade [25, 65]. This scenario is identical in many respects to the 'soft explosive' model of Phillips [15] except for the timing of the divergence of Xenarthra and Afrotheria.

Possible confounding factors

Branch lengths are contingent on both the fitted data and the model of evolution that guides that process. In ensuring that all morphological traits were binary, we avoided conflict between the limitations of currently implemented models and the nature of the - in particular morphological – data. While altering somewhat the composition of the dataset, this different treatment of characters is far from unusual, albeit falling on the 'splitting' end, whereas the matrix from which this analysis derives favoured 'lumping' of characters. Each character remains as logically independent as in the original matrix, with any upweighting of character states due to *de facto* non-independence of characters (no two characters are entirely independent) still satisfying the requirements of coding of morphological data. If characters transformed in this way were to all be synapomorphies of the same clade, it is possible that the morphological branch lengths would be confounded by this treatment, but in our data set this is not the case. Moreover, failing to account for ascertainment bias correctly can result in explosively long branches [47] and is likely to be far worse than any error introduced by any characters with a suboptimal coding format. Missing data in total evidence analyses does appear to be a problem with respect to fossils lacking the entirety of the genomic data [46], but we have heavily sampled morphology for our extant taxa, obviating some of that issue. For reconstruction of molecular branch lengths, this missing partition is not a problem, as fossils only contribute morphological information. Missing data in estimating total morphological rates of evolution is a possible concern; we do not know, for example, whether there was substantial unseen soft tissue evolution in the Cretaceous as our data set does not include soft tissue characters. As such, the results here can conservatively be said to hold true for the subset of identifiable skeleto-dental characters in our data matrix. Biases introduced from differing patterns of evolution within preserved and absent data partitions are important factors that warrant further investigation to add nuance to our interpretations here. Identifying patterns in the evolution of ecologically meaningful genes, such as the recent identification of parallel evolution of chitinase genes near the K-Pg boundary [66] will be particularly fruitful in this regard.

It is clear from our results that morphological to molecular branch length ratios vary substantially across the placental tree (Figure 1A), meaning that morphological and molecular data evolve, as hypothesised, in a decoupled fashion. Concentrating on the branches that constitute the diversification of the extant

orders and those more basal branches, there are consistent patterns over the sample of 1,000 trees as to which branches exhibit especially high or low ratios.

If results from the molecular dating analyses of dos Reis *et al*. [2] or Meredith *et al*. [11] are more accurate than those based on cal3 and comparable methods, the increase in morphological to molecular rate ratio cannot be explained by the end-Cretaceous mass extinction. The fossil record is complete enough in terms of both abundance and quality of fossils [20, 21] such that we should expect to have found Cretaceous placentals if they did exist, although Foote *et al*. [20] note three hypotheses that could explain the discrepancy between their results and postulated missing evolutionary history of eutherian mammals. Their first hypothesises posits that "Cretaceous members of the modern eutherian orders are preserved and described, but they are not recognized because they are so primitive and lack most diagnostic features", with the prediction that "both morphological evolution be largely decoupled from lineage splitting and molecular evolution and that eutherians experienced much lower rates of morphological change through the Cretaceous than during the Cenozoic".

Our previous work [10] demonstrated independence of morphological evolution from lineage splitting and lower rates of morphological change through the Cretaceous than the Cenozoic. Here we demonstrate substantial decoupling of morphological and molecular evolution. Our results suggest that we should expect ordinal diversification to have included substantial morphological divergence, making misidentification of Cretaceous stem members of placental orders as stem eutherians extremely unlikely. However, stem members of placental superorders are more plausibly misidentified as stem eutherians due to low relative and absolute rates of morphological evolution on those branches.

If the issue is in distinguishing between early crown-group placentals and their close stem relatives, the question remains as to why these taxa tend to fall outwith the crown in most cladistic analyses, rather than being ambiguous. Two explanations, neither excluding the other, exist. Firstly, a greater proportion of symplesiomorphies than synapomorphies, as a result of low rates of evolution, and secondly, the phenomenon of stemward slippage [67], which is thought to be a bigger problem in mammals than in other clades [68].

Late Cretaceous mammals, including leptictids and cimolestids, have typically been resolved outwith the crown group [1, 8, 19], but both groups have at some time been allied with crown group orders from comparative anatomy [69, 70] and are among the best candidates for recovered but unidentified Cretaceous placentals. Intriguingy, an undescribed specimen of *Gypsonictops*, a leptictid, has been recovered from the Turonian [71]. Several further taxa are reasonably well supported as members of the crown group, including the purported ungulate *Protungulatum* and the early primate *Purgatorius*, both of which are frequently recovered as stem-group placentals [8].

Structured biases in geographic preservation are still possible, so a short fuse cannot be entirely ruled out solely on the basis of these results, but even under these models of diversification, the discrepancy between the observed and predicted emergence of morphologically derived placental mammals is

substantially reduced when the relationship between morphological and molecular rate is accounted for across the placental phylogeny.

The identification of regions in the tree with higher or lower relative rates of morphological evolution reconciles several aspects of the enduring conflict between fossil and molecular data with respect to the placental radiation. Primarily, it allows discussion of patterns of rate evolution without needing to invoke the more controversial aspects of the timetrees, and demonstrates that conflict between these data sources is expected given their decoupled evolution. When several hypotheses of the timing of placental mammal diversification are invoked, it goes further. It explains why no conclusive crown placental mammal is known from the Cretaceous – palaeontologists might not expect to see much morphological difference between late stem and early crown Placentalia. It allows rapid morphological evolution in the aftermath of the extinction event without requiring concomitant unreasonable increases in mutation rate. In sum, Cretaceous and early Paleocene fossils contain vital information about morphological character evolution that, when taken into account in an appropriate manner, explains much of the controversy surrounding the timing and nature of the diversification of the placental mammals.

Data
All data and scripts used for this paper have been archived at DRYAD. doi:10.5061/dryad.2kp7vr8

Author Contributions
TJDH collected the morphological data; AUT prepared the genomic data; TJDH and HFG conducted the RAxML analysis, and TJDH subsequent analyses; AG, ZY, and TJDH conceived of the project; AG, ZY, and MdR directed and supervised the project.

Acknowledgements
We thank Judy Galkin and Eileen Westwig (American Museum of Natural History, New York), Guillaume Billet (Musée National d'Histoire Naturelle, Paris), Jack Ashby (Grant Museum, London), and Roberto Portela Miguez (Natural History Museum, London) for access to specimens, and to Mike Lee and three anonymous reviewers for their comments on this manuscript. This work was funded by Leverhulme Grant RPG-2014/364 to AG, ZY, and TJDH.

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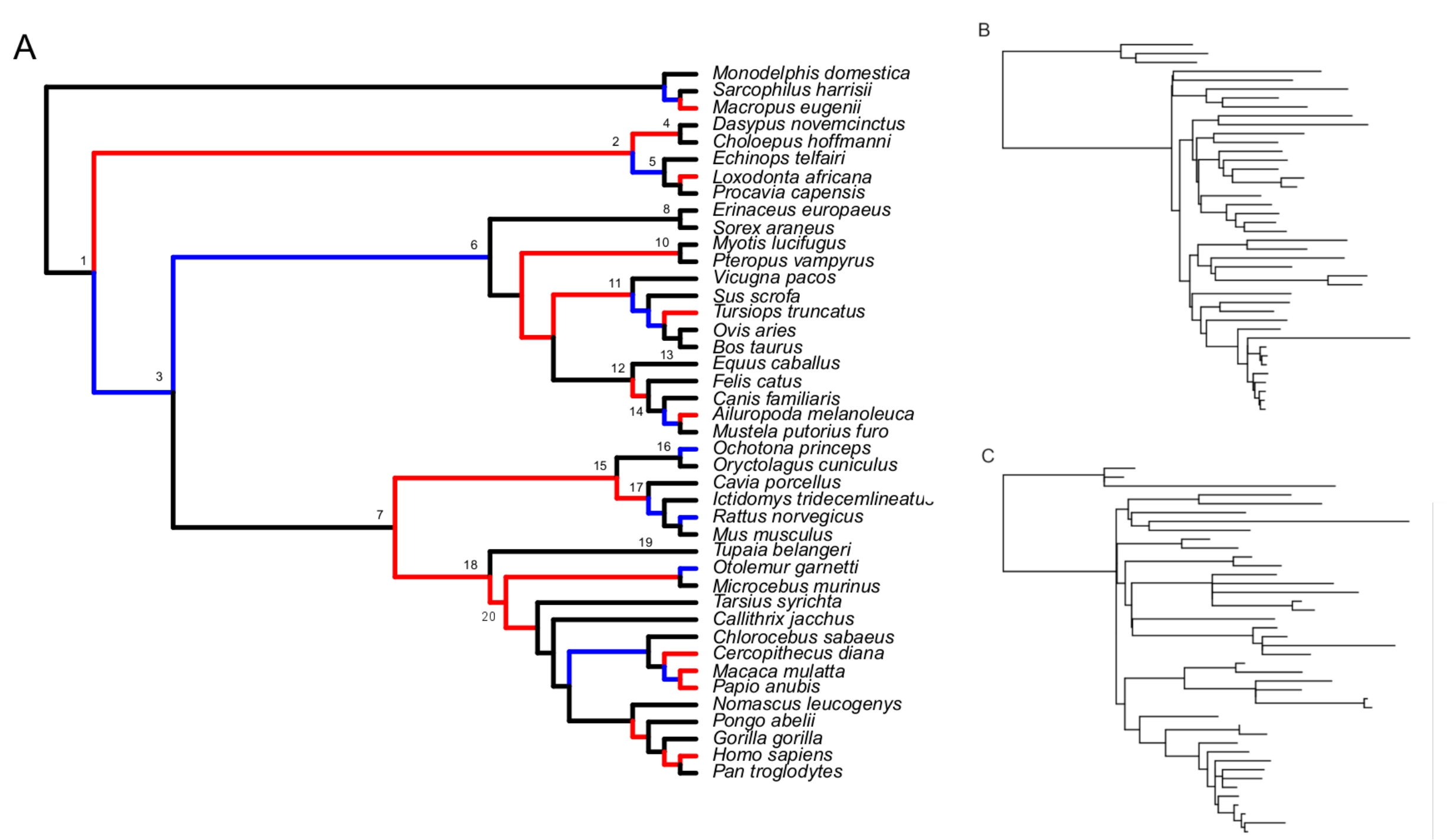
FIGURE LEGENDS

Figure 1: The phylogeny of extant placentals. A – Topology of extant placentals indicating branches with high (red) or low (blue) ratios of morphological:molecular evolution. Node numbers indicate clades, as follows: 1 – Placentalia, 2 – Atlantogenata, 3 – Boreoeutheria, 4 – Xenarthra, 5 – Afrotheria, 6 – Laurasiatheria, 7 – Euarchontoglires, 8 – Eulipotyphla, 9 – Scrotifera, 10 – Chiroptera, 11 – Artiodactyla, 12 – Pegasoferae, 13 – Perissodactyla, 14 – Carnivora, 15 – Glires, 16 – Lagomorpha, 17 – Rodentia, 18 – Euarchonta, 19 – Scandentia, 20 - Primates. B – Averaged molecular partition branch lengths. C – Averaged morphological partition branch lengths.

Figure 2: Rates of morphological and molecular evolution through time. A - Relative rate of morphological and molecular evolution through time according to four major dating hypotheses for placental mammal nodes, as follows: (i) Halliday and Goswami 2016, derived from a stochastic model and fossil occurrence data [10], (ii) Phillips 2016, derived from molecular data and incorporating strong assumptions about fossil calibrations [15], (iii) Meredith *et al*. 2011, a maximum likelihood relaxed clock model [13], and (iv) dos Reis *et al*. 2012, dated using a Bayesian approach and phylogenomic data set [12]. Although molecular-derived dates still imply an increase in morphological evolution prior to the end-Cretaceous mass extinction, the fact that this is not associated with the origin of Placentalia substantially reduces the conflict between the observations from fossil data and molecular-derived dates. Curves are presented excluding Xenarthra, because the exceptionally high rate ratios leading to that node overwhelm the signal from other branches of the phylogeny; versions including Xenarthra are available as SI Figure 1A-D. B – Absolute morphological (dotted line) and molecular (dashed line) rates of evolution through time for the four timetrees. In all but that of Halliday *et al*. 2016 [10], estimated absolute molecular rates undergo few major shifts over time. We note that even in the Halliday *et al*. 2016 timetree, which has the shortest branch durations, molecular rates are not excessively high near the KPg boundary. All molecular-derived trees imply some degree of morphological diversification during the Cretaceous that has not been observed, although the Cretaceous spike in the Phillips [15] tree represents the origin of Xenarthra, expected to have occurred in the undersampled southern continents.

Figure 3: Majority rule consensus tree of all maximum likelihood topologies generated across the 1,000 replicates for 57 extant and 191 extinct therian taxa.

Node annotations are internode certainties across all conflicting bipartitions (the ICA of Salichos *et al*. [72]). Support for most divisions within Placentalia is generally poor.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Macropus eugenii
Dasypus novemcinctus
Choloepus hoffmanni
Echinops telfairi
Loxodonta africana
Procavia capensis
Erinaceus europaeus
Sorex araneus
Myotis lucifugus
Pteropus vampyrus
Vicugna pacos
Sus scrofa
Tursiops truncatus
Ovis aries
Bos taurus
Equus caballus
Felis catus
Canis familiaris
Ailuropoda melanoleuca
Mustela putorius furo
Ochotona princeps
Oryctolagus cuniculus
Cavia porcellus
Ictidomys tridecemlineatus
Rattus norvegicus
Mus musculus
Tupaia belangeri
Otolemur garnetti
Microcebus murinus
Tarsius syrichta
Callithrix jacchus
Chlorocebus sabaeus
Cercopithecus diana
Macaca mulatta
Papio anubis
Nomascus leucogenys
Pongo abelii
Gorilla gorilla
Homo sapiens
Pan troglodytes
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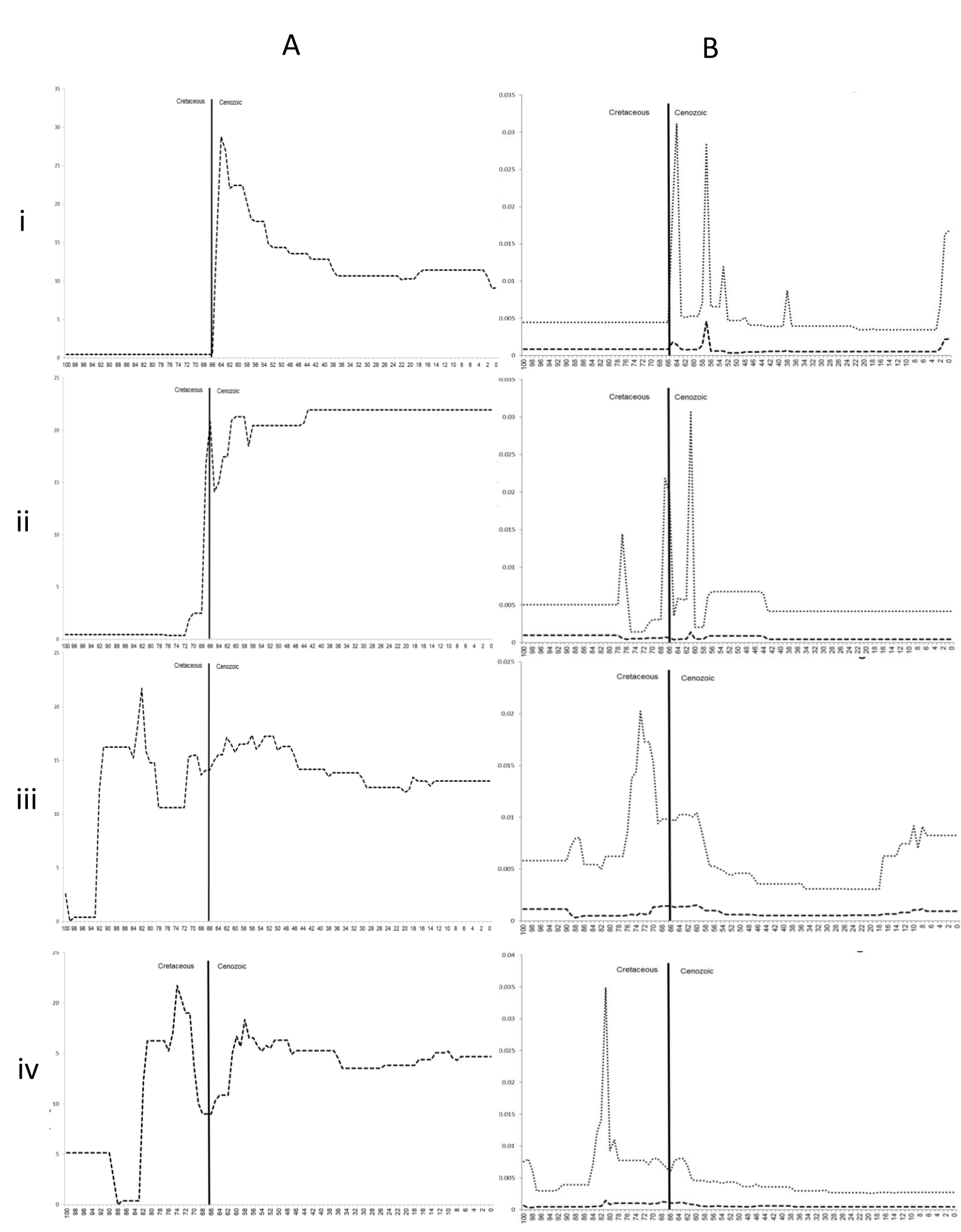
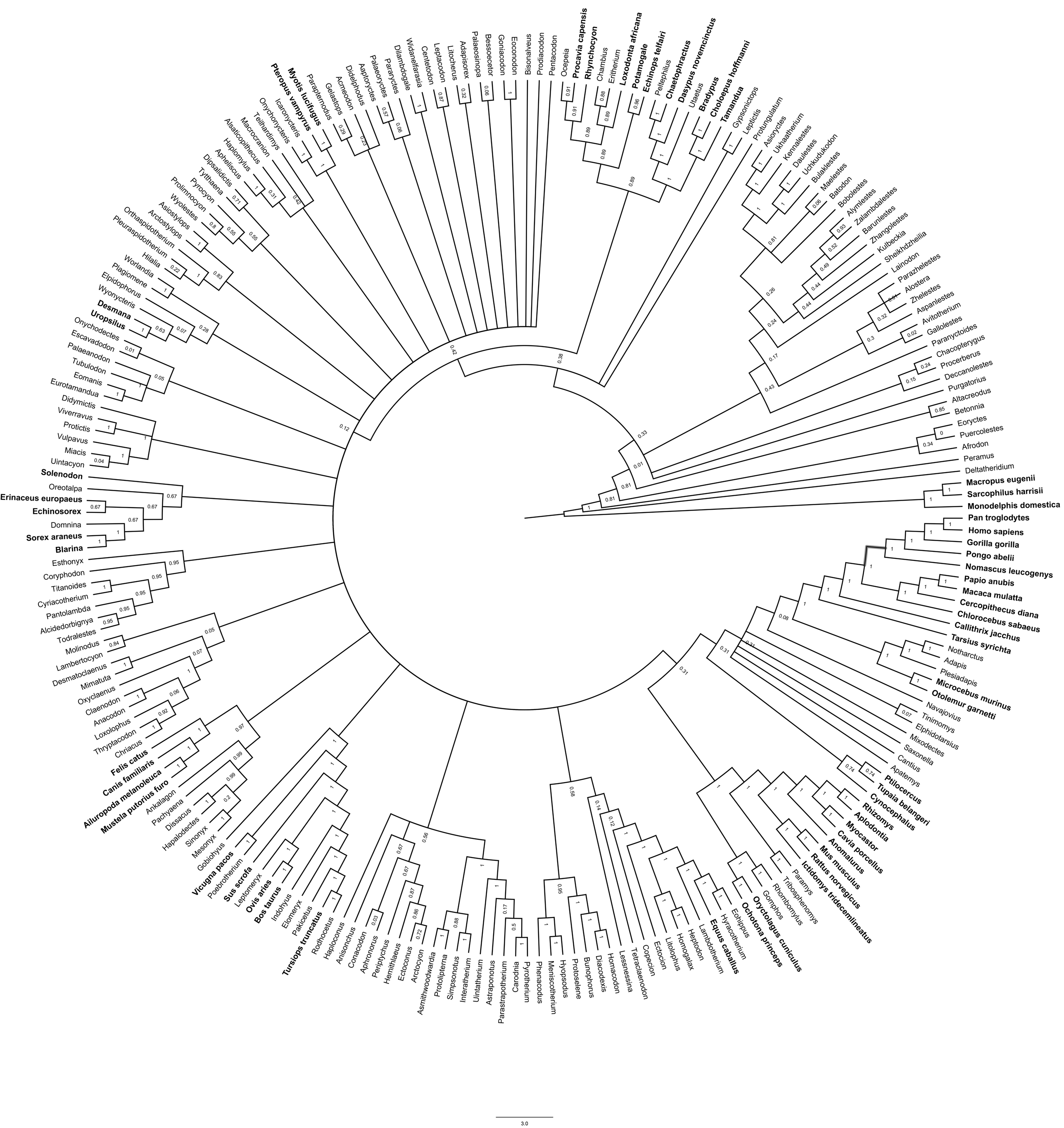

A
B
i
ii
iii
iv
Cretaceous
Cenozoic


Pteropus vampyrus
Myotis lucifugus
Desmana
Uropsilus
Solenodon
Erinaceus europaeus
Echinosorex
Sorex araneus
Blarina
Felis catus
Canis familiaris
Ailuropoda melanoleuca
Mustela putorius furo
Vicugna pacos
Sus scrofa
Ovis aries
Bos taurus
Tursiops truncatus
Procavia capensis
Rhynchocyon
Loxodonta africana
Potamogale
Echinops telfairi
Chaetophractus
Dasypus novemcinctus
Bradypus
Choloepus hoffmanni
Tamandua
Macropus eugenii
Sarcophilus harrisii
Monodelphis domestica
Pan troglodytes
Homo sapiens
Gorilla gorilla
Pongo abelii
Nomascus leucogenys
Papio anubis
Macaca mulatta
Cercopithecus diana
Chlorocebus sabaeus
Callithrix jacchus
Tarsius syrichta
Microcebus murinus
Otolemur garnetti
Ptilocercus
Tupaia belangeri
Cynocephalus
Rhizomys
Aplodontia
Myocastor
Cavia porcellus
Anomalurus
Mus musculus
Rattus norvegicus
Ictidomys tridecemlineatus
Oryctolagus cuniculus
Ochotona princeps
Equus caballus
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